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(54) Title: THERAPEUTIC USE OF PYRIDOXINE PYRROLIDONE CARBOXYLATE

(57) Abstract

Administration of pyridoxine pyrrolidone carboxylate enables to inhibit the formation of ethyl esters of free fatty acids and accumulation thereof in animal organs, as well as to compensate enzymatic function alterations connected with free fatty acids and esters thereof.

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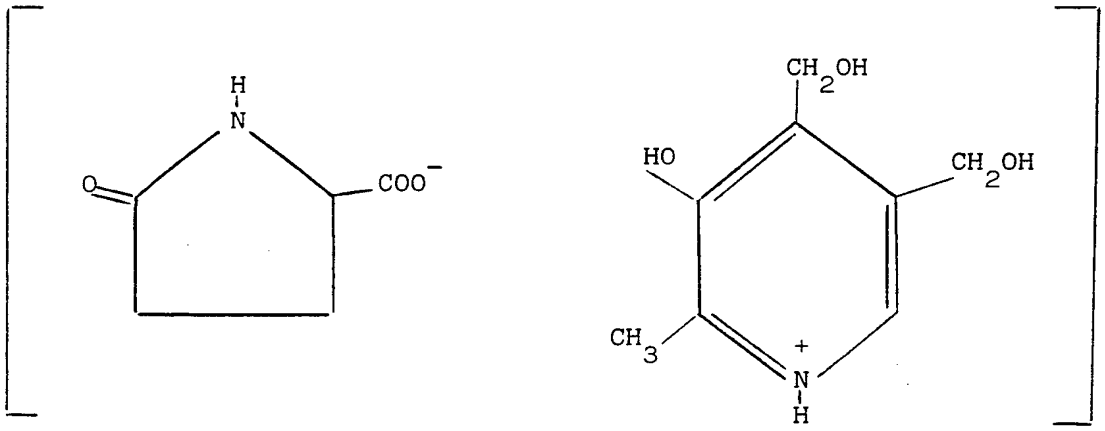
Therapeutic use of pyridoxine pyrrolidone carboxylate.

The present invention has as the object to provide a new therapeutic use of a known drug, namely pyridoxine 5-oxo-2-pyrrolidone carboxylate, also known as 5-hydroxy-6-methyl-3,4-pyridinemethanol 5-oxo-2-pyrrolidone carboxylate of the formula:



Pyridoxine, known also under the name of B6 vitamin, finds several therapeutic uses, among which the treatment of acute alcoholic intoxication states has been proposed in the past. However, this therapeutic indication has been subsequently abandoned, since it did not receive experimental confirmation.

On the contrary, the compound of the present invention finds also application in treating acute alcoholic intoxication caused by ethyl alcohol (Italian Patent No. 1.131.856).

The object of this invention is a novel therapeutic use of the same compound, i.e. pyridoxine 5-oxo-2-pyrrolidone carboxylate.

Free fatty acids and esters thereof, according to recent studies, have important effects in the human organism and, particularly, several negative or harmful effects, due to their accumulation in tissues, such as heart, brain, kidney, liver and plasma, have been noted.

As far as liver is concerned, for example, they are responsible of the so-called steatosis.

The ethyl esters of free fatty acids are, in turn, one of the main metabolites of the acids themselves and tend to accumulate in concentrations that might reach levels up to 100 micromoles in the myocardium, with a half life of nearly 16 hrs.

This means that they persist at tissue level even after the factors promoting these accumulations are eliminated, as, for example, the ethyl alcohol action.

The biological importance of these esters was recently demonstrated and can be summarized as follows:

(1) reduction of the respiratory liver and heart rhythm of the isolated mitochondrions;

(2) induction of a disorder state at cellular membrane level, probably due to a lipase of the membrane itself which decomposes said ester to free fatty acids;

(3) inhibition, even at low concentrations, of the hepatic protein synthesis as well as of the triacylglycerol lipase.

Essentially, it can be considered that the free fatty acids esterification represents a vehicle of toxic states.

It has now been found, and is the object of the present invention, that the administration of pyridoxine pyrrolidone carboxylate, that is, of the compound as above chemically characterized, allows to substantially decrease free fatty acids ethyl ester formation, with clear, favourable consequences.

It is, therefore, the object of the instant invention the use of pyridoxine 5-oxo-2-pyrrolidone carboxylate as active principle for inhibiting the free fatty acid ethyl esters, the therapeutic treatment including the active principle administration in oral dosages from 250 mg/day to 2500 mg/day, and in parenteral dosages from 250 mg/day to 1500 mg/day.

Some of the experimental investigations that led to discover this specific property of the active principle, the use of which is the object of the present invention, will now be illustrated.

Owing to experimentation requirements, the pharmacological researches have been carried out by means of ethanol administration, since it can promote the formation and accumulation, in the above-indicated tissues and organs, of the afore-mentioned pyridoxine pyrrolidone carboxylate esters.

Wistar rats of 250-300 g weight have been randomly divided in groups of six animals each.

The first group of animals was orally administered with a 20% ethanol solution at a 2 g/kg dose for a week.

The second group was administered as the first group, except that each day, for all the seven days of the parenteral treatment, received 60 mg/kg of pyridoxine pyrrolidone carboxylate in crystalline form, 30 minutes before the ethanol solution was administered.

The third group was used as a control group and was administered with a physiological solution.

At the end of the week treatment, the animals were sacrificed and the various tissues were analyzed.

Tables 1 and 2 report the measuring data of fatty acid ethyl ester, obtained in vivo, in the heart, plasma, liver, kidney and brain, respectively.

The heart is the organ showing the highest levels of ethyl esters (6.96 nmoles of total esters/mg of proteins). Stearate, linoleate, linolenate and arachidonate are the mostly present chemical species of ethanol esterified fatty acids.

As the decreasing contents of total esters, the analyzed organs showed the following sequence:

heart - kidney - brain - plasma - liver

The treatment with the active principle of the invention produces a significant reduction of the contents of total esters formed, particularly in the kidney, brain, heart and plasma (80-60%).

In the following tables, E means ethanol, while M stands for methdoxine, this is pyridoxine pyrrolidone carboxylate.

Table 1

Formation of fatty acid ethyl ester after ethanol consumption in the presence and with methadoxine administration.

nmoles / mg protein

FAEE	HEART		PLASMA	
	E	E-M	E	E-M
14:0	0.758+0.07		0.035+0.01	
14:1	0.303+0.06		0.018+0.01	
16:0	0.386+0.03	0.161+0.03	0.100+0.04	
16:1	0.140+0.02		0.149+0.04	
18:0	1.218+0.88	0.062+0.02	0.083+0.02	0.047+0.09
18:1			0.231+0.03	
18:2	0.601+0.06	0.018+0.03	0.182+0.06	
18:3	0.996+0.06	0.462+0.06		0.160+0.03
20:0	0.796+0.07		0.236+0.02	
20:4	1.760+0.05	0.068		
22:0				
Total	6.867	0.772	1.044	0.207

E: ETHANOL (2g/Kg)

M: · METHADOXINE (600 mg/Kg)

Table 2

Formation of fatty acid ethyl ester after ethanol consumption in the presence and with methadoxine administration.

nmoles M mg protein

	LIVER		KIDNEY		BRAIN	
FAEE	E	E-M	E	E-M	E	E-M
14:0	0.097+0.02		0.049+0.01	0.034+0.01	0.037+0.01	0.071+0.02
16:0	0.040+0.01	0.600+0.07	0.036+0.01		0.036+0.01	0.017+0.01
18:0	0.220+0.06	0.064+0.02	1.10 +0.06	0.014+0.01	0.121+0.02	0.081+0.03
18:1	0.112+0.04		0.149+0.02		0.126+0.04	0.016+0.01
18:2	0.330+0.03		0.186+0.03		0.130+0.02	
18:3	0.222+0.04	0.020+0.01	0.467+0.02	0.014+0.01	0.233+0.03	0.115+0.06
20:0	0.086+0.02	0.061+0.01	0.118+0.02	0.013+0.01	0.684+0.07	
20:4	0.039+0.01					
Total	1.146	0.664	2.105	0.076	1.26	0.300

E: ETHANOL (2g/Kg)

M: METHADOXINE (600 mg/Kg)

Table 3, in turn, shows the effect of ethanol solution administration on the GSH-S-transferase activity.

As it can be seen, the activity of this enzyme appears to be strengthened by the ethanol administration in the liver, kidney, heart and, particularly, in the brain (87,6%).

The concurrent methadoxine treatment induces an almost total normalisation of this enzyme activity, with the exception of liver, where it results to be still more strengthened.

Table 3

Effect of ethanol on the Glutation-S-Transferase activity with and without Methadoxine

	DCNB–GSH TRASFERASE nmoles / min / mg protein		
	CONTROL	E	E–M
LIVER	10.1 ± 1.2	16.67 ± 1.3	35.86 ± 1.6
KIDNEY	1.50 ± 0.6	4.5 ± 0.2	2.12 ± 0.2
BRAIN	1.40 ± 0.7	11.34 ± 1.7	4.60 ± 1.2
HEART	0.92 ± 0.1	3.32 ± 0.4	1.09 ± 0.3

E = ETHANOL (2g/Kg)

M = METHADOXINE (600 mg/Kg)

In turn, Table 4 illustrates the effect of the ethanol administration on the activity decrease of the GSH reductase, which occurs in all tested organs, except for brain, where it is found to be strengthened.

This inhibition, with methadoxine, is nearly totally compensated in the plasma, liver, kidney and reduced in the heart.

Table 4

Effect of acute ethanol on the Glutatione Reductase with and without Methadoxine

	GSH REDUCTASE nmoles / min / mg protein		
	Controls	E	E–M
PLASMA	0.386 ± 0.026	0.237 ± 0.08	2.21 ± 0.03
RBC	3.58 ± 0.42	1.71 ± 1.1	2.79 ± 0.01
LIVER	60.96 ± 9.2	36.72 ± 3.2	61.46 ± 3.3
KIDNEY	80.36 ± 2.3	62.67 ± 1.7	71.30 ± 4.8
BRAIN	8.20 ± 2.9	18.3 ± 5.9	12.60 ± 6.7
HEART	14.64 ± 1.8	8.07 ± 0.9	12.40 ± 2.0

E = ETHANOL (2g/Kg)

M = METHADOXINE (600 mg/Kg)

Finally, Table 5 shows the ethanol effect in terms of decrease of GSH levels in all examined organs as compared with the control animals, while an increase of GSSG is recorded. The concurrent methadoxine interperitoneal administration shows to protect the organs from such a reduction, especially referred to brain.

Table 5

Effect of acute ethanol on oxydized and reduced Glutation with and without Methdoxine treatment

	nmoles / mg protein					
	CONTROLS		ETHANOL		ETHANOL+METHADOXINE	
	GSH	GSSG	GSH	GSSG	GSH	GSSG
PLASMA	0.325+0.064	0.019+0.008	0.177+0.034	0.026+0.009	0.62 +0.083	0.107+0.022
RBC	11.1 + 1.3	0.12+0.10	8.9 + 0.8	0.553+0.075	8.77 +0.9	0.343+0.111
LIVER	118.1+14.0	0.231+0.098	41.5+7.8	0.336+0.064	82.34+9.8	0.463+0.131
KIDNEY	9.49 + 2.1	0.056+0.015	6.61+0.96	0.252+0.088	9.58 + 1.1	0.172+0.06
BRAIN	35.5+9.0	0.260+0.078	25.47+11.2	0.412+0.037	54.02+7.7	0.221+0.017

From the previous experimental results it is proved that pyridoxine pyrrolidone carboxylate is able to perform an effective therapeutic action in preventing the fatty acid ethyl ester formation and the accumulation thereof in animal organs.

At the same time, this active principle appears to be useful in compensating unbalances of enzymatic factors, which are seemingly the origin of tumoral phenomena bound with the same rousing factors as for example the ethanol, of the free accumulation of fatty acids and of their main metabolites.

According, therefore, to the present invention, the administration of this active principle by the normal administration means and known dosages for normal uses is to be suggested, when the free fatty acid ester formation, accumulation of the acids themselves and alteration of enzymatic functions bound to said free fatty acid action, such as GSH-S-transferase and GSH-reductase, must be controlled and compensated for.

As regards the preparation of the pharmaceutical compositions using the active principle of this invention and dosages thereof, reference is made to the already known pharmaceutical compositions for the control and therapy of acute alcoholic intoxication.

CLAIMS

1. Use of pyridoxine 5-oxo-2-pyrrolidone carboxylate to inhibit the free fatty acid accumulation in patients' body and of the formation of ethyl esters of the same acids, as well as to offset the alterations of enzymatic functions connected with said free fatty acid action.

2. Use of the pyridoxine 5-oxo-2-pyrrolidone carboxylate according to claim 1, characterized in that it is intended for the epatic steatosis therapy.

3. Use of pyridoxine 5-oxo-2-pyrrolidone carboxylate according to claim 1, characterized in that the parenteral administration of the active principle is to be foreseen at dosages from 250 mg/day to 1500 mg/day .

4. Use of pyridoxine 5-oxo-2-pyrrolidone carboxylate according to claim 1, characterized in that the oral administration of the active principle is to be foreseen at dosages from 250 mg/day to 2500 mg/day.

5. Use of pyridoxine 5-oxo-2-pyrrolidone carboxylate according to claim 1 in the preparation of pharmaceutical compositions effective for the treatments, recited in claims 1 and 2.

INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/EP 94/00518

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR,A,2 485 372 (LABORATORI BALDACCI SPC) 31 December 1981 see the whole document ---	1-5
X	CLIN.TRIALS J. vol. 23, no. 5 , 1986 pages 289 - 292 L.CACCIATORE ET AL. 'METADOXINE PREVENTION OF ALCOHOL-INDUCED FATTY LIVER IN THE RAT' see the whole document ---	1-5
X	CLIN.TER. vol. 119, no. 2 , 31 October 1986 pages 133 - 141 L.SERGIACOMO ET AL. 'TRATTAMENTO DELL' EPATOSTEATOSI ALCOOLICA CON METADOXINA' see the whole document --- -/--	1-5

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLIN.TRIALS J. vol. 25, no. 3 , 1988 pages 220 - 226 L.CACCIATORE ET AL. 'METADOXINE TREATMENT OF FATTY LIVER ASSOCIATED WITH CHRONIC HEPATITIS' see the whole document ---	1-5
X	CLIN.THER. vol. 137, no. 1 , 15 April 1991 pages 29 - 38 A. MANCINELLA 'STEATOSI E FIBROSI: PRIMA TAPPA DEL DANNO EPATICO INDOTTO DALL' ABUSO CRONICO DI ALCOOL' see the whole document ---	1-5
X	INT.J.TISSUE REACT. vol. 12, no. 4 , 1990 pages 247 - 250 S. MARCHI ET AL. 'LIVER TRIGLYCERIDE ACCUMULATION AFTER CHRONIC ETHANOL ADMINISTRATION: A POSSIBLE PROTECTIVE ROLE OF METADOXINA AND UBIQUINONE' see the whole document ---	1-5
X	CHEMICAL ABSTRACTS, vol. 118, no. 9, 1 March 1993, Columbus, Ohio, US; abstract no. 76259s, E.F.KONOPLYA ET AL. 'Corrective activity of metadoxil in radiation-induced dyslipoproteinemia' see abstract & VESTSI AKAD. NAVUK BELARUSI, SER. BIYAL. NAVUK. no. 3-4 , 1992 pages 55 - 59 -----	1-5

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internatic Application No
PCT/EP 94/00518

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		AU-A-	7234981	07-01-82
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